Mimivirus Relatives in the Sargasso Sea

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Summary

The discovery and genome analysis of *Acanthamoeba polyphaga Mimivirus*, the largest known DNA virus, challenged much of the accepted dogma regarding viruses. Its particle size (>400 nm), genome length (1.2 million bp) and huge gene repertoire (911 protein coding genes) all contribute to blur the established boundaries between viruses and the smallest parasitic cellular organisms. Phylogenetic analyses also suggested that the Mimivirus lineage could have emerged prior to the individualization of cellular organisms from the three established domains, triggering a debate that can only be resolved by generating and analyzing more data. The next step is then to seek some evidence that Mimivirus is not the only representative of its kind and determine where to look for new Mimiviridae. An exhaustive similarity search of all Mimivirus predicted proteins against all publicly available sequences identified many of their closest homologues among the Sargasso Sea environmental sequences. Subsequent phylogenetic analyses suggested that unknown large viruses evolutionarily closer to Mimivirus than to any presently characterized species exist in abundance in the Sargasso Sea. Their isolation and genome sequencing could prove invaluable in understanding the origin and diversity of large DNA viruses, and shed some light on the role they eventually played in the emergence of eukaryotes.

Introduction

The discovery and genome sequence analysis of Mimivirus [1,2], the largest of the Nucleo-cytoplasmic Large DNA Viruses (NCLDV), challenged much of the accepted dogma regarding viruses. Its particle size (>400 nm), genome length (1.2 million bp) and extensive gene repertoire (911 protein coding genes) all contribute to blur the established boundaries between viruses and the smallest parasitic cellular organisms such as Mycoplasma or Nanoarchea [2]. In the universal tree of life, the Mimivirus lineage appears to define a new branch, predating the emergence of all established eukaryotic kingdoms [2]. Although this result is compatible with various hypotheses implicating ancestral DNA viruses in the emergence of eukaryotes [3-5], it requires confirmation from additional data. An urgent task is thus to convince ourselves that Mimivirus is not the sole representative of its kind (i.e. a viral counterpart to the platypus) and to provide some rational guidance as to where to begin the search for eventual new *Mimiviridae*.

Mimivirus was serendipitously discovered within *Acanthamoeba polyphaga*, a free-living ubiquitous amoeba, prevalent in aquatic environments. Phylogenetic analysis of the most conserved genes common to all nucleo-cytoplasmic large double-stranded DNA viruses (NCLDV) [6] positions Mimivirus as an independent lineage, roughly equidistant from the Phycodnaviridae (algal viruses) and Iridoviridae (predominantly fish viruses). Given the ecological affinity of these virus families for the marine environment, we have examined the sequence data set gathered through environmental microbial DNA sampling in the Sargasso Sea [7] to look for possible Mimivirus relatives.

Results

By comparing Mimivirus ORFs to the Sargasso Sea sequence data set and to all other publicly available sequences, 138 (15%) of the 911 Mimivirus ORFs were found to exhibit their closest match (Blastp E-values ranging from 10^{-74} to 10^{-4} [8]) to environmental sequences (see Materials and Methods). Even before the discovery of Mimivirus, increasingly complex large double-stranded DNA viruses have been isolated, in particular from unicellular algae. The genome analysis of these Phycodnaviruses revealed a variety of genes encoding enzymes from totally unexpected metabolic pathways [9]. Mimivirus added more unexpected genes (such as translation system components [2]) to this list. As the gene repertoire of these large viruses and the gene content of cellular organisms become increasingly comparable, we have to be cautious in the interpretation of environmental/metagenomics sequence data. To focus our study on environmental organisms most likely to be viruses, we limited further analyses to

Mimivirus homologues member of the NCLDV core gene sets [2,6]. These core genes are subdivided into four classes from the most (class I) to least (class IV) evolutionarily conserved [6]. Seven of 10 Mimivirus Class I core genes have their closest homologues in the Sargasso Sea data. This is also the case for 3 of 7 class II core genes, 3 of the 13 class III core genes and 7 of the 16 Class IV core genes (Table 1). Overall, 43% of Mimivirus core genes have their closest homologues in the Sargasso Sea data set. To further assess the viral nature of these unknown microbes, we studied the phylogenetic relationships between the corresponding Mimivirus proteins, their Sargasso Sea homologues, and the closest homologues in other NCLDVs (see Materials and Methods). Figure 1 a-c exhibits three independent phylogenic trees computed using the MEGA3 software [10] for Mimivirus ORFs R449 (unknown function), R429 (unknown function) and L437 (putative virion packaging ATPase). Figure 1a shows that the closest environmental R449 homologues cluster with Mimivirus separately from the known phycodnaviruses, while other Sargasso Sea homologues cluster in a way suggesting the presence of a new clade distinct from Phycodnaviridae. The tree based on R429 and L437 (Fig. 1b, c) similarly suggests the presence of close Mimivirus relatives not belonging to the Phycodnaviridae or Iridoviridae clades.

Another piece of evidence substantiating the existence of an unknown Mimivirus relative in the Sargasso Sea is the discovery of contigs built from the data that contain multiple genes with a high degree of similarity to Mimivirus genes. A spectacular case is illustrated in Figure 2. Here, a 4.5 kb scaffold (See supplementary information) exhibits 4 putative ORFs. When compared to the whole nr database, each of them has as a best match 4 distinct Mimivirus ORFs: thiol oxidoreductase R368 (29% identical, E-value<10^{-9}), NTPase-like L377 (25% identical, E-value<10^{-20}), unknown function L375 (34% identical, E-value<10^{-30}), and DNA repair enzyme L687 (40% identical, E-value<10^{-62}). Moreover, the gene order is conserved for three of them (R368, L375, L377). Such colinearity is rarely observed between viral genomes except for members of the same family. Unfortunately, the sequences of these genes are not conserved enough to allow the construction of informative phylogenic trees that would include other NCLDV orthologues.

As of today, genes encoding capsid proteins are among the most unequivocal genes of viral origin. Except for cases of integrated proviral genomes, no cellular homologues of viral capsid proteins have ever been found. During our study, the closest homologues of Mimivirus capsid proteins were found to be capsid protein genes of environmental origin. For example, Mimivirus capsid protein (R441) was found to be 48.5% identical to an unknown environmental sequence, when it is only 36.2% identical to the major capsid protein Vp49 of

Chlorella virus CVG-1, its best match among known viruses (Figure 3). As the environmental capsid protein sequence also shares 44.5% identical residues with the CVG-1 Vp49, the corresponding uncharacterized virus appears to lie at an equal evolutionary distance from the *Mimiviridae* and the *Phycodnaviridae*.

Discussion

Our results predict that DNA viruses of 0.1 to 0.8 microns in size exist in the Sargasso Sea that are evolutionarily closer to Mimivirus than to any presently characterized species. These viruses are abundant enough to have been collected by environmental sampling. It is actually expected that many novel viruses will be encountered in natural waters in which they constitute the most abundant microrganisms [11, 12]. There might be as many as 10 billion virus particles per litre of ocean surface waters [13]. Interestingly, the specialized literature abounds of descriptions of large virus-like particle associated with algae [e.g. 14,15,16], or various marine protists [17,18]. With the exception of Phycodnaviruses [19], the genomic characterization of these viruses has not been attempted. Guided by the results presented here, their isolation and genome sequencing could prove invaluable in understanding the diversity of DNA viruses and the role they eventually played in the evolution of eukaryotes.

Materials and Methods

The protocols used to collect Sargasso Sea environmental micro-organisms and generate DNA sequences from these samples has been described elsewhere (7). The data analyzed here correspond to "bacteria-sized" organisms that have passed through 3 μm filters and been retained by 0.8 μm to 0.1 μm filters. Mimivirus-like particles (0.8-0.4 μm) belong in this range.

Database similarity searches were performed using the Blast suite of programs (8) (default options) as implemented on the www.giantvirus.org web server and as implemented at The Institute for Genomic Research. Final similarity searches were performed on the non-redundant peptide sequence databases (nr) and environmental data (env-nr) downloaded from the National Institute for Biotechnology Information ftp server (ftp.ncbi.nlm.nih.gov/blast/db/) on March 14, 2005. To avoid missing potential better matches with annotated virus ORFs, all Mimivirus ORFs exhibiting a best match (blosum62 scoring scheme) in env-nr were also searched against all DNA virus genomes using TblastN (peptide query against translated nucleotide sequence). The comprehensive list of Mimivirus ORFs exhibiting a best match in the env-nr database is given in the supplementary material section.

Phylogenetic analyses were conducted using MEGA version 3.0 (10) (option: Neighbor joining, 250 pseudo-replicates, and gaps handled by pairwise deletion). Tree branches were condensed for bootstrap values < 50%.

Only Mimivirus ORFs with best matching homologues in DNA viruses and belonging to the nucleo-cytoplasmic large DNA virus core gene set (2, 6) were analyzed in detail. These ORFs (and matching status) are listed in Table 1 from the most conserved (type 1, in yellow) to the least conserved (type 2 in green, type 3 in blue, and type 4 in white). Phylogenetic analyses were limited to viral homologues and environmental sequences exhibiting a reciprocal best match relationship with the corresponding Mimivirus ORF (putative orthologues) (YES in the rightmost column). The three cases (red lines in Table 1) exhibiting the best bootstrap values are shown in Figure 1. Cases of complex relationships, for instance due to the presence of many paralogues (e.g. capsid proteins), are also indicated. These cases of non-reciprocal best matches are frequent (i.e. the closest homologue of a Mimivirus ORFs being an environmental sequence, but the latter sequence exhibiting a better match with a different ORF in the nr database).

Two environmental sampling contigs - contig IBEA_CTG_1979672 (AACY01022731, GI:44566181) and contig IBEA_CTG_1979673 (AACY01022732, GI:44566179) - are linked in a 4,465 bp scaffold (scaffold IBEA_SCF=2208413) found to contain four ORFs with strong matches to Mimivirus peptides (R368, L377, L375, and L687). The three colinear ORFs (R368, L377, L375) are found on one contig while the orthologue to Mimivirus ORF L687 is found in the second contig. It is conceivable that the lack of colinearity for this fourth ORF is due to an assembly error.

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Table 1. Matching Status of Mimivirus core genes (type 1 to 4).

ORF#	Definition	Best score in nr	Best score in DNA viruses	Best score in Sargasso Sea	Status	Reciprocal Best match
L206	Helicase III / VV D5	167- virus	167	214	Best ENV	YES
R322	DNA pol (B family) extein	207	167	238	Best ENV	YES
L437	**A32 virion packaging ATPase**	**169 - virus**	**169**	**191**	**Best ENV**	**YES**
L396	VV A18 helicase	200 -virus	200	187	-	
L425	Capsid protein	119 - virus	117	142	Best ENV	complex
R439	Capsid protein	164 - virus	159	173	Best ENV	complex
R441	Capsid protein	137 - virus	147	209	Best ENV	complex
R596	E10R-Thiol oxidoreductase	104 -virus	105	119	Best ENV	YES
R350	VV D6R - helicase	170 -virus	170	102	-	
R400	F10L - prot. Kinase	86 -virus	86	58	-	
R450	A1L-transcr factor	52 -virus	47	65	Best ENV	
R339	TFII-transcr. factor	62	42	66	Best ENV	
L524	MuT-like NTP PP-hydrolase	40	38	39	-	
L323	Myristoylated virion prot. A	43	42	40	-	
R493	PCNA	92	87	154	Best ENV	YES
L312	Small Ribonucl. reduct	341	338	310	-	
R313	Large Ribonucl. reduct	766	741	740	-	
R429	**PBCV1-A494R-like**	**152-virus**	**152**	**216**	**Best ENV**	**YES**
L37	BroA, KilA-N	123-virus	124	65	-	
R382	mRNA–capping enz.	86	78	166	Best ENV	YES
L244	RNA pol. sub 2 (Rbp2)	727	416	508	-	
R501	RNA pol. sub.1 (Rpb1)	805	415	520	-	
R195	ESV128- Glutaredoxin	50	39	49	-	
R622	S/Y phosphatase	75	73	65	-	
R311	CIV193R BIR domain	68	44	51	-	
L65	Virion memb. prot	44	44	-	-	
R480	Topoisomerase II	902	717	367	-	
L221	Topoisomerase I bacterial	528	35	516	-	
R194	Topoisomerase I pox-like	188	100	145	-	
L364	SW1/SNF2 helicase	70-virus	70	72	Best ENV	YES
L4 + 7 par	N1R/P28 DNA binding prot	123-virus	124	72	-	
L540	Pre-mRNA helicase - splicing	256	136	214	-	
L235	RNA pol subunit5	69	38	50	-	
R354	Lambda-type exonuclease	69-virus	69	154	Best ENV	YES
R343	RNAse III	129	112	131	Best ENV	YES
R141	GDP mannose 4,6-dehydratase	294	68	252	-	
L258	Thymidine kinase	151	140	124	-	
L271 + par	Ankyrin repeats (66 paralogs)	179	152	192	Best ENV	complex
R325	Metal-dependent hydrolase	69-virus	69	105	Best ENV	YES
L477	Cathepsin B	226	43	47	-	
R497	Thymidylate synthase	278	242	217	-	
R449	**Uncharacterized prot.**	**69-virus**	**69**	**129**	**Best ENV**	**YES**
R303	NAD-dependent DNA ligase	270-virus	270	228	-	
L805	MACRO domain	36	33	-	-	
R571 L446	Patatin-like phospholipase	105	80	122	Best ENV	YES
R301	Uncharacterized prot.	48-virus	48	65	Best ENV	YES

Figure legend

Figure 1. Phylogenetic evidence of uncharacterized Mimivirus relatives. (a) Neighbor-joining (NJ) clustering (see Materials and Methods) of Mimivirus R449 ORF with its best matching (≈35% identical residues) environmental homologues (noted Sargasso1 to Sargasso6 according to their decreasing similarity) and closest viral orthologues (28% identical). **(b)** NJ clustering of Mimivirus R429 ORF with its best matching (≈50% identical) environmental homologues (noted Sargasso1 to Sargasso5) and closest viral orthologues (35% identical). **(c)** NJ clustering of Mimivirus putative virion packaging ATPase L437 with its best matching (≈45% identity) environmental homologues (Sargasso1 and Sargasso2) and closest viral orthologues (34% identical). Abbreviations: Phyco: Phycodnavirus; PBCV: *Paramecium bursaria* chlorella virus 1; EsV: *Ectocarpus siliculosus* virus; FsV: Feldmannia sp. virus; HaV: Heterosigma akashiwo virus; Irido: Iridovirus; LCDV: Lymphocystis disease virus 1; Frog: Frog virus 3; Amby: *Ambystoma tigrinum stebbensi* virus; Rana: *Rana tigrina ranavirus*; Chilo: Chilo iridescent virus. Bootstrap values larger than 50% are shown. Branches with lower values were condensed.

Figure 2. Organization of four Mimivirus ORF best matching homologues in a 4.5 kb environmental sequence scaffold (approximately to scale). The three colinear Mimivirus homologues are in green. Unmatched ORF extremities are indicated by dots. The two diagonal lines indicate where the two contigs are joined on the scaffold.

Figure 3. Partial 3-way alignment (N-terminus region) of Mimivirus capsid protein (R441) with it best matching homologues in the NR and Environmental sequence databases. The Mimivirus R441 protein shares 83/229 (36.2%) identical residues (colored in red or blue) with the major capsid protein Vp49 of Chlorella virus CVG-1 and 111/229 (48.5%) identical residues (indicated in red or green) with the N-terminus of a capsid protein from an unknown large virus sampled from the Sargasso Sea (Accession: EAD00518). On the other hand, the CVG-1 Vp49 and the Sargasso Sea sequence share 44.5 % identical residues. By comparison, the CVG-1 Vp49 protein share 72% of identical residue with PBCV-1 Vp54, its best matching homologue among known phycodnaviruses.

Figure 1

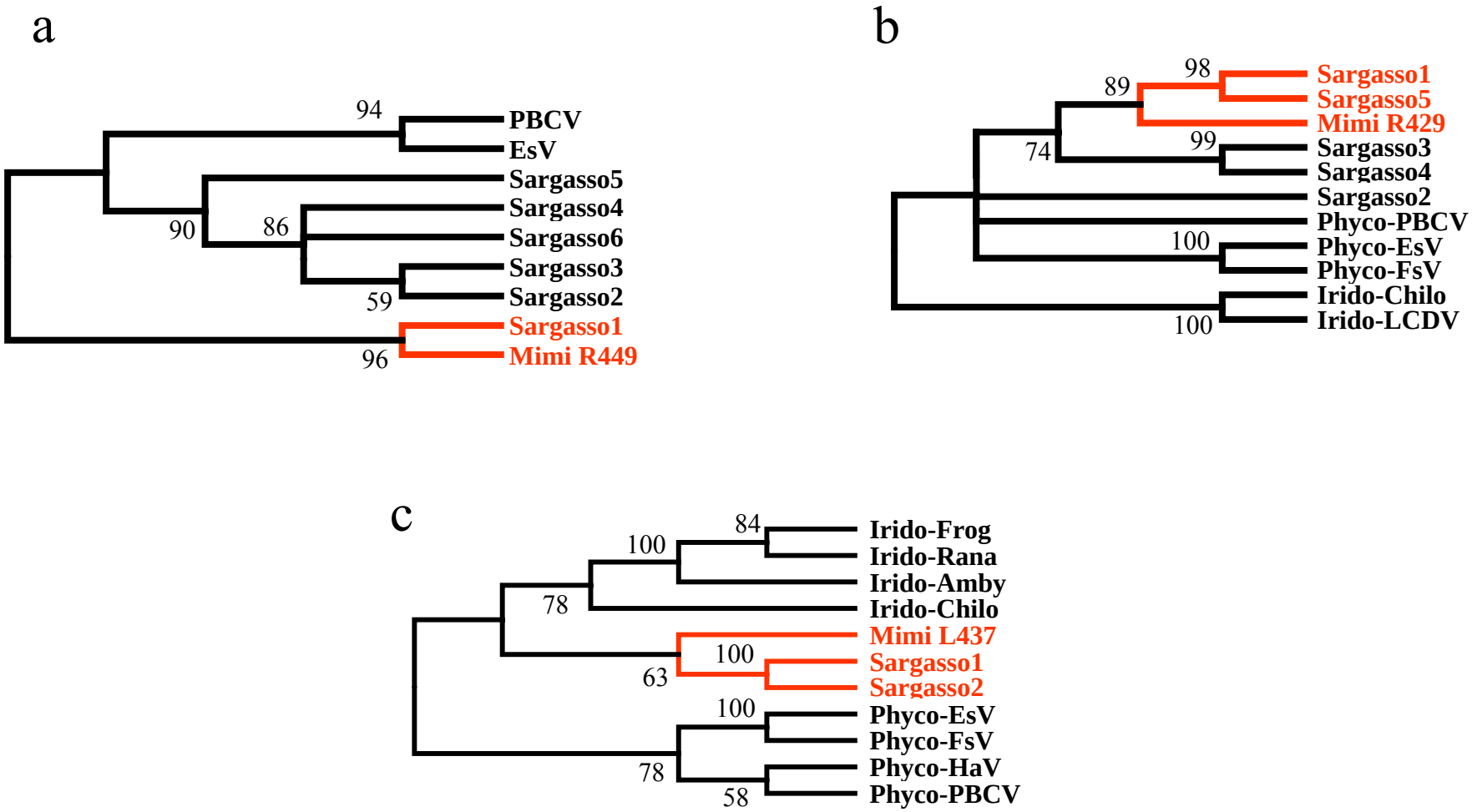
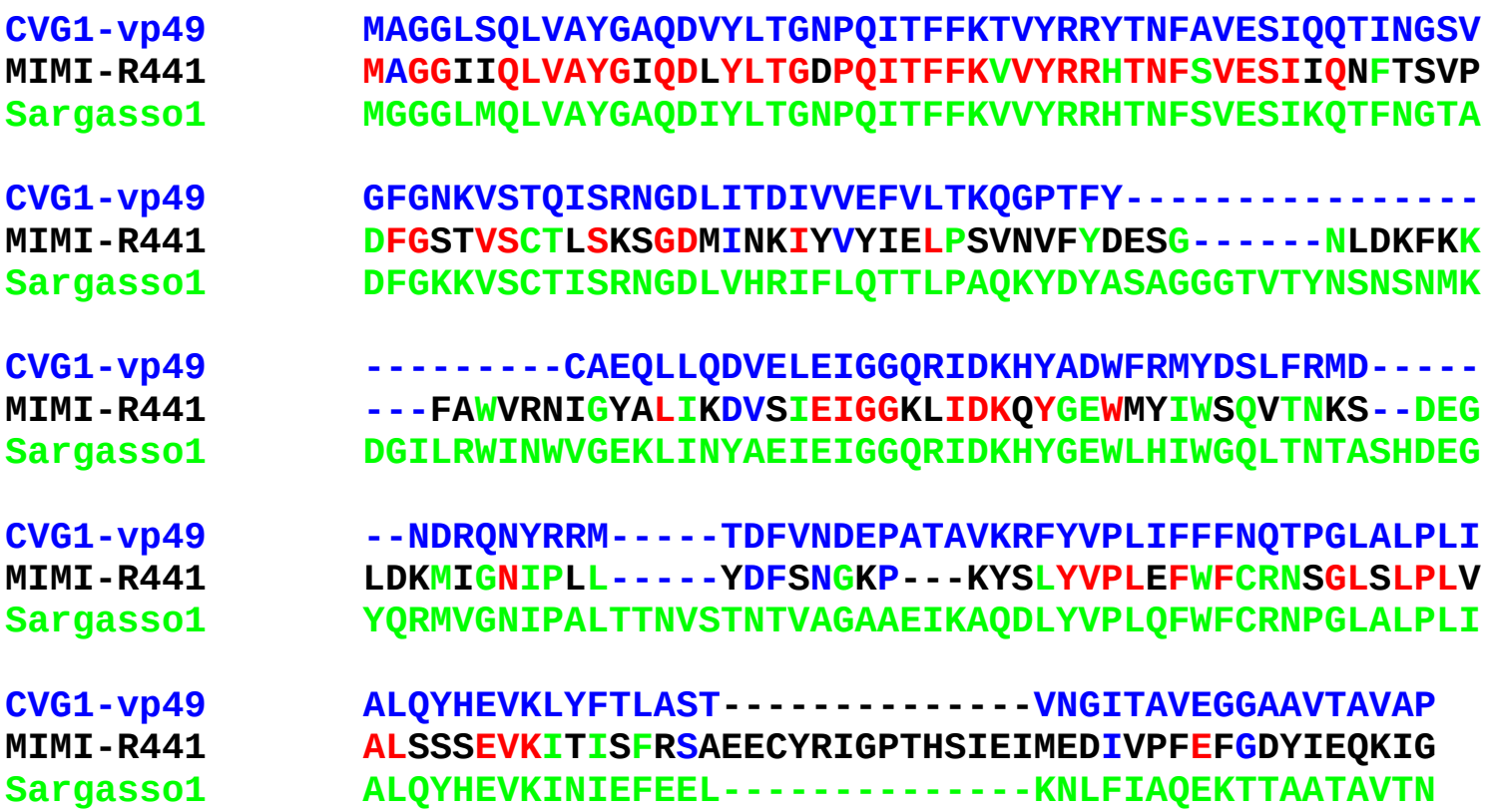

a
94
PBCV
EsV
Sargasso5
Sargasso4
90
86
Sargasso6
Sargasso3
59
Sargasso2
Sargasso1
96
Mimi R449
b
98
89
Sargasso1
Sargasso5
Mimi R429
99
74
Sargasso3
Sargasso4
Sargasso2
Phyco-PBCV
100
Phyco-EsV
Phyco-FsV
Irido-Chilo
100
Irido-LCDV
c
84
100
Irido-Frog
Irido-Rana
Irido-Amby
78
Irido-Chilo
Mimi L437
100
63
Sargasso1
Sargasso2
100
Phyco-EsV
Phyco-FsV
78
Phyco-HaV
58
Phyco-PBCV

Figure 2

Figure 3


CVG1-vp49 MAGGLSQLVAYGAQDVYLTGNPQITFFKTVYRRYTNFAVESIQQTINGSV
MIMI-R441 MAGGIIQLVAYGIQDLYLTGDPQITFFKVVYRRHTNFSVESIIQNFTSVP
Sargasso1 MGGGLMQLVAYGAQDIYLTGNPQITFFKVVYRRHTNFSVESIKQTFNGTA
CVG1-vp49 GFGNKVSTQISRNGDLITDIVVEFVLTKQGPTFY---------------
MIMI-R441 DFGSTVSCTLSKSGDMINKIYVYIELPSVNVFYDESG------NLDKFKK
Sargasso1 DFGKKVSCTISRNGDLVHRIFLQTTLPAQKYDYASAGGGTVTYNSNSNMK
CVG1-vp49 ---------CAEQLLQDVELEIGGQRIDKHYADWFRMYDSLFRMD-----
MIMI-R441 ---FAWVRNIGYALIKDVSIEIGGKLIDKQYGEWMYIWSQVTNKS--DEG
Sargasso1 DGILRWINWVGEKLINYAEIEIGGQRIDKHYGEWLHIWGQLTNTASHDEG
CVG1-vp49 --NDRQNYRRM-----TDFVNDEPATAVKRFYVPLIFFFNQTPGLALPLI
MIMI-R441 LDKMIGNIPLL-----YDFSNGKP---KYSLYVPLEFWFCRNSGLSLPLV
Sargasso1 YQRMVGNIPALTTNVSTNTVAGAAEIKAQDLYVPLQFWFCRNPGLALPLI
CVG1-vp49 ALQYHEVKLYFTLAST--------------VNGITAVEGGAAVTAVAP
MIMI-R441 ALSSSEVKITISFRSAEECYRIGPTHSIEIMEDIVPFEFGDYIEQKIG
Sargasso1 ALQYHEVKINIEFEEL--------------KNLFIAQEKTTAATAVTN

Supplementary material

List of Mimivirus ORFs exhibiting a best match in the env-nr database

Mimi ORF	Best Env-nr Match ID	Score	E-value	Best nr Match ID	Score	E-value
L18	gi|43710841|gb|EAF10508.1|	137.1	4.7e-31	gi|51244078|ref|YP_063962.1|	106.7	2.7e-21
L25	gi|42923128|gb|EAB28610.1|	169.1	5e-41	gi|34876677|ref|XP_214012.2|	163.3	1.1e-38
L75	gi|43124292|gb|EAC28017.1|	51.6	1.1e-05	gi|13794513|gb|AAK39888.1|	51.22	5.5e-05
L93	gi|42923128|gb|EAB28610.1|	177.9	1.6e-43	gi|40740451|gb|EAA59641.1|	156.8	1.5e-36
L100	gi|42923128|gb|EAB28610.1|	119	1.4e-25	gi|40740451|gb|EAA59641.1|	117.9	1.2e-24
L102	gi|44215848|gb|EAH75003.1|	46.98	0.00017	gi|19703853|ref|NP_603415.1|	41.2	0.035
R106	gi|43776260|gb|EAF42614.1|	139	8.1e-32	gi|37676542|ref|NP_936938.1|	107.1	1.4e-21
L111	gi|43296731|gb|EAD14168.1|	46.21	0.0006	gi|16805307|ref|NP_473335.1|	41.2	0.077
L113	gi|43770287|gb|EAF39597.1|	50.45	8.8e-06	gi|23498951|emb|CAD51029.1|	43.9	0.003
R118	gi|44281621|gb|EAI17665.1|	187.6	1.6e-46	gi|56965351|ref|YP_177083.1|	43.51	0.014
R132	gi|42849963|gb|EAA92307.1|	98.6	4.3e-20	gi|33864966|ref|NP_896525.1|	61.62	2.1e-08
R135	gi|44399015|gb|EAI99687.1|	99.37	1.4e-19	gi|48769830|ref|ZP_00274174.1|	97.06	2.7e-18
L136	gi|43375980|gb|EAD52870.1|	211.5	9.9e-54	gi|56708496|ref|YP_170392.1|	107.5	7.9e-22
R139	gi|42931622|gb|EAB32840.1|	57	1.8e-07	gi|30409752|gb|AAP32727.1|	55.45	2e-06
L143	gi|43788785|gb|EAF48931.1|	101.7	8e-21	gi|54031509|ref|ZP_00363643.1|	53.91	7.4e-06
L174	gi|43709436|gb|EAF09806.1|	48.52	0.0001	gi|13358136|ref|NP_078410.1|	45.44	0.003
L177	gi|43660484|gb|EAE85189.1|	47.75	0.00021	gi|56526423|emb|CAH77752.1|	44.67	0.007
L193	gi|43116621|gb|EAC24307.1|	138.7	1.7e-31	gi|59889773|emb|CAH19128.1|	110.2	2.6e-22
L206	gi|44511459|gb|EAJ77190.1|	210.7	2e-53	gi|13358409|ref|NP_078717.1|	163.7	1.1e-38
L207	gi|43582640|gb|EAE46140.1|	115.2	1.9e-24	gi|13358409|ref|NP_078717.1|	101.3	1.1e-19
L208	gi|43154232|gb|EAC42788.1|	54.3	6.8e-07	gi|23509124|ref|NP_701792.1|	44.67	0.002
L215	gi|43198765|gb|EAC64922.1|	45.44	9.8e-05	gi|30249486|ref|NP_841556.1|	43.9	0.001
R240	gi|43054398|gb|EAB93597.1|	83.57	9.3e-15			
L250	gi|43527692|gb|EAE18541.1|	133.7	3.8e-30	gi|17136758|ref|NP_476888.1|	121.3	7.9e-26
L251	gi|44049491|gb|EAG84927.1|	263.5	8.5e-69	gi|13357908|ref|NP_078182.1|	251.5	1.4e-64
R267	gi|42923128|gb|EAB28610.1|	114	2.9e-24	gi|40740517|gb|EAA59707.1|	111.7	5.7e-23
L279	gi|42977843|gb|EAB55869.1|	46.21	0.00082	gi|23508131|ref|NP_700801.1|	45.05	0.007
R296	gi|44416870|gb|EAJ11899.1|	138.7	6.8e-32	gi|48845024|ref|ZP_00299314.1|	132.9	1.5e-29
R299	gi|43651599|gb|EAE80654.1|	82.42	3.4e-15	gi|46440930|gb|EAL00231.1|	80.88	3.7e-14
L300	gi|44143264|gb|EAH36242.1|	59.31	1.5e-08	gi|50292185|ref|XP_448525.1|	54.3	1.7e-06
R301	gi|42942338|gb|EAB38176.1|	64.7	1.8e-09	gi|15042460|gb|AAK82240.1|	48.14	0.00068
L315	gi|42953235|gb|EAB43598.1|	88.97	5.4e-17	gi|56964473|ref|YP_176204.1|	70.86	5.9e-11

L318	gi|44268792|gb|EAI09019.1|	214.2	1.5e-54	gi|33416901|gb|AAH55597.1|	160.2	1e-37
L320	gi|43010993|gb|EAB72269.1|	101.3	8.6e-21	gi|30691953|ref|NP_174343.2|	67.01	6.9e-10
R322	gi|44500797|gb|EAJ70042.1|	230.7	1.1e-58	gi|1655695|emb|CAA93738.1|	160.2	7.5e-37
R325	gi|42880571|gb|EAB07497.1|	98.6	3.4e-20	gi|2738426|gb|AAB94453.1|	58.15	1.8e-07
R339	gi|43154902|gb|EAC43116.1|	60.46	7.5e-09	gi|17136888|ref|NP_476967.1|	58.92	7.6e-08
R341	gi|42929462|gb|EAB31721.1|	122.1	1.6e-26	gi|9628175|ref|NP_042761.1|	72.02	7.5e-11
R343	gi|43750296|gb|EAF29561.1|	131.3	2.3e-29	gi|30021937|ref|NP_833568.1|	129	4.6e-28
R354	gi|43821272|gb|EAF65062.1|	154.1	3.4e-36	gi|9631735|ref|NP_048514.1|	68.94	5.8e-10
R355	gi|43665722|gb|EAE87837.1|	121.3	1.1e-26	gi|450711|emb|CAA50819.1|	52.37	2.4e-05
L364	gi|42987215|gb|EAB60545.1|	72.02	1.7e-11	gi|4049749|gb|AAC97709.1|	63.54	2.4e-08
R366	gi|43171483|gb|EAC51308.1|	255	5.5e-66	gi|50302815|ref|XP_451344.1|	144.1	5.6e-32
R368	gi|43105861|gb|EAC18976.1|	113.6	4.3e-25	gi|13177431|gb|AAK14575.1|	73.94	1.1e-12
L371	gi|42973284|gb|EAB53609.1|	113.6	7.7e-24	gi|40556241|ref|NP_955326.1|	109.8	4.5e-22
L374	gi|43006294|gb|EAB69978.1|	58.15	6.6e-08	gi|49528793|emb|CAG62455.1|	37.35	0.452
L375	gi|44363842|gb|EAI74667.1|	131.3	1.3e-29	gi|450699|emb|CAA50807.1|	72.4	2.9e-11
L377	gi|43715140|gb|EAF12631.1|	141.7	4.2e-32	gi|37722439|gb|AAP33184.1|	127.9	2.6e-27
R378	gi|43174352|gb|EAC52755.1|	53.53	1.2e-06	gi|56489446|emb|CAI03544.1|	49.68	6.4e-05
R382	gi|44465210|gb|EAJ45951.1|	166	2.1e-39	gi|6319713|ref|NP_009795.1|	85.89	1.2e-14
R383	gi|43262281|gb|EAC96836.1|	69.32	6e-11	gi|17137638|ref|NP_477413.1|	51.6	5.1e-05
L388	gi|44253396|gb|EAH98417.1|	77.8	1.1e-13	gi|56961986|ref|YP_173708.1|	75.48	2.1e-12
L396	gi|43615322|gb|EAE62377.1|	173.3	5.1e-42	gi|13177345|gb|AAK14489.1|	167.5	1.1e-39
R398	gi|43115033|gb|EAC23498.1|	120.6	1.3e-26	gi|46226486|gb|EAK87480.1|	62.39	1.7e-08
R409	gi|43205815|gb|EAC68464.1|	52.37	2.4e-06	gi|37725924|gb|AAO38040.1|	38.89	0.097
R411	gi|43523940|gb|EAE16653.1|	78.57	1.8e-13	gi|3116125|emb|CAA18875.1|	77.8	1.2e-12
L417	gi|44253353|gb|EAH98387.1|	102.1	1.3e-20	gi|9632055|ref|NP_048844.1|	59.31	4e-07
R418	gi|44628310|gb|EAK59119.1|	122.9	4.8e-28	gi|11498373|ref|NP_069601.1|	121.3	5.8e-27
L425	gi|44004806|gb|EAG60442.1|	142.5	8e-33	gi|4587052|dbj|BAA76601.1|	119.8	2.2e-25
L426	gi|43035635|gb|EAB84418.1|	68.55	5e-11	gi|46241679|gb|AAS83064.1|	63.16	7.8e-09
R429	gi|43593604|gb|EAE51661.1|	216.5	4.1e-55	gi|9632061|ref|NP_048850.1|	152.5	2.9e-35
R430	gi|42973834|gb|EAB53885.1|	53.91	1.6e-06	gi|9632061|ref|NP_048850.1|	46.21	0.001
L432	gi|44173673|gb|EAH52299.1|	151.4	5.4e-36	gi|48730983|ref|ZP_00264729.1|	146.4	6.6e-34
R435	gi|43287470|gb|EAD09576.1|	66.63	4.8e-10	gi|23481897|gb|EAA18039.1|	48.52	0.00054
L437	gi|44215544|gb|EAH74836.1|	177.6	1.1e-43	gi|16151622|dbj|BAB69884.1|	157.1	6.1e-37
R439	gi|43111515|gb|EAC21807.1|	173.7	4.6e-42	gi|4587052|dbj|BAA76601.1|	164.5	1.1e-38
R440	gi|43011410|gb|EAB72486.1|	90.12	2.9e-16	gi|23510178|ref|NP_702844.1|	78.95	2.7e-12
R441	gi|43269509|gb|EAD00518.1|	209.9	4.8e-53	gi|3341805|gb|AAC27492.1|	137.9	9.4e-31
R443	gi|44226821|gb|EAH81210.1|	63.16	1e-09	gi|56470459|gb|EAL48116.1|	52.37	6e-06
R445	gi|43027882|gb|EAB80631.1|	50.06	4.1e-05	gi|34397716|gb|AAQ66777.1|	48.14	0.00061

L446	gi|42912213|gb|EAB23180.1|	95.52	7.2e-19	gi|47569527|ref|ZP_00240206.1|	84.73	5e-15
R447	gi|43125924|gb|EAC28806.1|	60.46	2.9e-09	gi|9632052|ref|NP_048841.1|	46.98	0.00014
R449	gi|43476579|gb|EAD93014.1|	122.9	1.1e-26	gi|13177377|gb|AAK14521.1|	62.39	6.9e-08
R450	gi|42883300|gb|EAB08831.1|	48.91	8.6e-05	gi|38683713|gb|AAR26889.1|	40.05	0.157
L451	gi|44145880|gb|EAH37686.1|	51.22	2.2e-05	gi|23612730|ref|NP_704269.1|	45.82	0.004
R453	gi|43138976|gb|EAC35259.1|	74.33	2e-12	gi|23481840|gb|EAA17997.1|	51.99	4.2e-05
L454	gi|43184473|gb|EAC57805.1|	72.79	2.7e-11	gi|16805082|ref|NP_473111.1|	70.09	7.1e-10
R468	gi|43479103|gb|EAD94286.1|	46.98	0.00017			
L471	gi|44521596|gb|EAJ83983.1|	48.91	0.0001	gi|23510142|ref|NP_702808.1|	44.67	0.008
R472	gi|43137277|gb|EAC34419.1|	111.7	7.2e-23	gi|33414605|gb|AAL38220.2|	63.54	9.3e-08
L479	gi|44350833|gb|EAI65324.1|	82.03	5.2e-15	gi|52788091|ref|YP_093919.1|	70.48	6e-11
L483	gi|42923128|gb|EAB28610.1|	161	1.9e-38	gi|42555731|gb|EAA78537.1|	157.5	8.3e-37
L485	gi|44073294|gb|EAG98118.1|	48.52	2.8e-05	gi|23509456|ref|NP_702123.1|	41.2	0.015
R489	gi|43155682|gb|EAC43508.1|	67.78	8e-11	gi|9631920|ref|NP_048709.1|	47.75	0.00032
L491	gi|44049493|gb|EAG84928.1|	61.62	7.8e-09	gi|56473336|gb|EAL50770.1|	48.52	0.00026
R493	gi|43155685|gb|EAC43510.1|	153.3	4.7e-36	gi|18726|emb|CAA39239.1|	88.58	5.7e-16
L496	gi|43924053|gb|EAG16876.1|	67.4	1.5e-10	gi|60468406|gb|EAL66411.1|	51.99	2.6e-05
R502	gi|44245809|gb|EAH92811.1|	46.21	0.00033	gi|23612359|ref|NP_703939.1|	38.12	0.343
L504	gi|43757624|gb|EAF33258.1|	51.22	2.1e-05	gi|23619172|ref|NP_705134.1|	40.05	0.192
L507	gi|44275242|gb|EAI13417.1|	99.37	3.3e-20	gi|9632038|ref|NP_048827.1|	88.97	1.7e-16
R508	gi|43850909|gb|EAF79670.1|	70.09	3.3e-11	gi|46440772|gb|EAL00074.1|	43.9	0.01
R512	gi|43084896|gb|EAC08655.1|	107.8	5.3e-23	gi|34333239|gb|AAQ64394.1|	68.55	1.3e-10
L539	gi|44612196|gb|EAK47432.1|	60.08	2.5e-08	gi|48095940|ref|XP_394563.1|	53.91	6.9e-06
R555	gi|44074387|gb|EAG98725.1|	221.1	6.6e-56	gi|48477311|ref|YP_023017.1|	129	1.4e-27
R568	gi|44444139|gb|EAJ31047.1|	82.03	2.2e-14	gi|42782567|ref|NP_979814.1|	78.18	1.3e-12
R569	gi|43851406|gb|EAF79912.1|	46.59	0.00037	gi|14247226|dbj|BAB57617.1|	42.36	0.027
R571	gi|42912213|gb|EAB23180.1|	103.2	2.9e-21	gi|54303145|ref|YP_133138.1|	87.43	6.5e-16
R592	gi|43246588|gb|EAC88951.1|	70.48	8.4e-11	gi|19173110|ref|NP_597661.1|	67.01	3.8e-09
L593	gi|44438438|gb|EAJ27151.1|	128.3	6e-29	gi|54029386|ref|ZP_00361528.1|	114.4	3.4e-24
R595	gi|43331658|gb|EAD31349.1|	97.44	1.6e-19	gi|42553185|gb|EAA76028.1|	90.12	9.8e-17
R596	gi|43382223|gb|EAD56003.1|	119.8	2.9e-26	gi|13177431|gb|AAK14575.1|	104.4	5e-21
R604	gi|43049485|gb|EAB91155.1|	47.37	0.00028	gi|23484531|gb|EAA19833.1|	44.28	0.01
L620	gi|42912213|gb|EAB23180.1|	57.77	2e-07	gi|19705025|ref|NP_602520.1|	55.45	3.9e-06
R640	gi|44283592|gb|EAI18988.1|	124.8	2.1e-27	gi|37519905|ref|NP_923282.1|	112.8	3.4e-23
R648	gi|44283592|gb|EAI18988.1|	167.5	2e-40	gi|37519905|ref|NP_923282.1|	122.1	3.8e-26
R654	gi|43291944|gb|EAD11806.1|	97.44	4.3e-19	gi|32263328|gb|AAP78373.1|	84.34	1.5e-14
R667	gi|43769283|gb|EAF39072.1|	55.07	2.1e-07	gi|52841308|ref|YP_095107.1|	50.83	9.6e-06
L687	gi|44566182|gb|EAK15053.1|	244.6	9.5e-64	gi|46097173|gb|EAK82406.1|	214.9	3.2e-54

R689	gi|44068613|gb|EAG95539.1|	150.6	1.2e-35	gi|24378003|gb|AAN59275.1|	144.1	4.4e-33
L690	gi|44283592|gb|EAI18988.1|	152.5	6e-36	gi|37519905|ref|NP_923282.1|	104.4	7.4e-21
R693	gi|43163235|gb|EAC47212.1|	119.4	8.9e-27	gi|49235486|ref|ZP_00329554.1|	115.5	3.2e-25
L716	gi|44016225|gb|EAG66729.1|	69.32	2.4e-11	gi|15921096|ref|NP_376765.1|	66.24	7.4e-10
R730	gi|44083879|gb|EAH03977.1|	199.5	4.8e-50	gi|6466376|gb|AAF12958.1|	123.2	1.7e-26
R753	gi|43500977|gb|EAE05248.1|	85.89	1.7e-15	gi|46447199|ref|YP_008564.1|	71.25	1.7e-10
R757	gi|43500977|gb|EAE05248.1|	73.17	9.5e-12	gi|46447096|ref|YP_008461.1|	65.86	6.2e-09
R758	gi|44662698|gb|EAK80724.1|	54.68	4.7e-07	gi|50755591|ref|XP_414809.1|	51.22	1.9e-05
R760	gi|43408903|gb|EAD65356.1|	97.44	3.4e-19	gi|20521133|dbj|BAA31672.2|	96.67	2.3e-18
R777	gi|42923128|gb|EAB28610.1|	95.52	6.3e-19	gi|18676694|dbj|BAB84999.1|	90.89	6.1e-17
L780	gi|43372155|gb|EAD50923.1|	280.4	1.3e-74	gi|56472969|gb|EAL50422.1|	237.7	3.7e-61
R791	gi|43884722|gb|EAF96312.1|	79.72	3.3e-14	gi|32419933|ref|XP_330410.1|	78.95	2.2e-13
R802	gi|44389434|gb|EAI92933.1|	59.69	2.7e-08	gi|25010522|ref|NP_734917.1|	57.77	3.9e-07
R815	gi|44467483|gb|EAJ47513.1|	109.4	9.5e-23	gi|52841586|ref|YP_095385.1|	107.8	1.1e-21
R832	gi|44335875|gb|EAI54742.1|	123.2	4.5e-27	gi|13475556|ref|NP_107120.1|	112.5	3.1e-23
R835	gi|43881322|gb|EAF94645.1|	87.43	1.8e-16	gi|24646968|ref|NP_650415.1|	84.34	5.8e-15
R837	gi|44398294|gb|EAI99192.1|	86.27	1e-15	gi|1845265|gb|AAB47805.1|	84.73	1.2e-14
R843	gi|44554385|gb|EAK06651.1|	53.53	2.9e-06			
R844	gi|43318044|gb|EAD24737.1|	83.57	1.3e-15	gi|53730512|ref|ZP_00151259.2|	76.64	6e-13
R846	gi|43713504|gb|EAF11838.1|	61.23	4.2e-09	gi|47214900|emb|CAG01031.1|	60.46	2.5e-08
R847	gi|44398294|gb|EAI99192.1|	74.71	2.4e-13	gi|28373837|pdb|1N0R|A	71.63	5.2e-12
R848	gi|42923128|gb|EAB28610.1|	110.5	1.5e-23	gi|58699292|ref|ZP_00374082.1|	98.98	1.7e-19
R850	gi|43710841|gb|EAF10508.1|	65.86	1.1e-09	gi|48834353|ref|ZP_00291368.1|	63.16	3e-08
R852	gi|43536538|gb|EAE22952.1|	115.9	2e-25	gi|33860982|ref|NP_892543.1|	99.37	7e-20
R853	gi|43166050|gb|EAC48610.1|	108.2	1.2e-23	gi|33860982|ref|NP_892543.1|	86.27	2.1e-16
R855	gi|44585905|gb|EAK28804.1|	120.6	2.4e-27	gi|33860982|ref|NP_892543.1|	101.3	6.2e-21
R877	gi|43313336|gb|EAD22405.1|	95.13	2.6e-19	gi|26990735|ref|NP_746160.1|	94.74	1.2e-18
L893	gi|44629372|gb|EAK59899.1|	62.77	1e-09	gi|57101548|ref|XP_541839.1|	59.31	2.7e-08
L894	gi|44600496|gb|EAK39086.1|	109.4	7.2e-23	gi|45684031|ref|ZP_00195462.1|	93.59	1.6e-17
R901	gi|42923128|gb|EAB28610.1|	162.5	5.5e-39	gi|42555731|gb|EAA78537.1|	157.1	9.1e-37